PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference S 10010 PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/11551	International filing date *(day/month/year)* 17.10.2003	Priority date *(day/month/year)* 18.10.2002
International Patent Classification (IPC) or both national classification and IPC C12N15/10		
Applicant SLONING BIOTECHNOLOGY GMBH et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 10 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
22.04.2004	07.02.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Loubradou-Bourges, N Telephone No. +49 89 2399-7342

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/11551

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-54 as originally filed

Sequence listings part of the description, Pages

1-23 as originally filed

Claims, Numbers

1-22 received on 27.12.2004 with letter of 23.12.2004

Drawings, Sheets

1/26-26/26 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 2, 9-10

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 2, 9-10

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☒ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1,3,4-7 part, 8, 11-18, 19-22 .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1, 3,4-7 part, 8, 11-18, 19-22
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1, 3,4-7 part, 8, 11-18, 19-22
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1, 3,4-7 part, 8, 11-18, 19-22
	No:	Claims	

2. Citations and explanations

see separate sheet

The IPER is restricted to the 3 inventions for which examinations fees were paid, namely :
- invention 1, comprising claims 1, 3, 4-7 partly, 8 partly
- invention 4, comprising claims 8 partly, 11-18
- invention 5, comprising claims 19-22

Reference is made to the following document :

D1: WO 00/75368 A (DIAVIR GMBH ;SCHATZ OCTAVIAN (DE)) 14 December 2000 (2000-12-14)

SECTION V

prior art :
D1 discloses a method for the manufacture of a nucleic acid molecule (named SPS method), that can be resumed as a method wherein
a) an oligonucleotide is immobilised on a matrix
b) a second free oligonucleotide is added
c) the oligonucleotides are ligated resulting in a immobilised ligation product
e) the ligation product is restricted
f) the resulting elongated oligonucleotide is separated from the matrix.

invention 1

Invention 1, as is described in independent claims 1, 3, 8 (part) and dependent claims thereof may be seen as three methods comprising more or less repetitions of the same basic steps as described in claim 1 a) to e).
The subject-matter of claim 1 relates to a method for manufacture of a nucleic acid molecule that can be resumed in a method wherein
a) a free oligonucleotide is provided
b) a second free oligonucleotide is provided
c) the first and the second oligonucleotides are ligated, generating a ligation product

d) the ligation product is immobilised to a surface
e) the immobilised ligation product is cut
f) to l) the steps of providing two free (elongated) oligonucleotides, ligating, immobilising, cutting is repeated.

The subject-matter of invention 1 differs substantially from the one disclosed in D1 in that the order of the steps is different. In invention 1, ligation occurs before immobilisation, whereas in D1 immobilisation of the first oligonucleotide occurs before ligation. This alternative was not described nor suggested in D1. Novelty is therefore acknowledged (Art.33(2) PCT). Comparative tests over D1 were provided with fig.12 and p.49 of the present application, showing an improvement of the method of the present application (named RLPS) over the method of the prior art, in particular with respect to side products. Inventive activity is therefore acknowledged (Art.33(3) PCT).

For the sake of completeness, it is noted that the alternative part of claim 8 related to in aiii) and in bv) and read "or the second (the fourth respectively) elongated oligonucleotide is immobilised to a surface... generating in both cases a first (second respectively) ligated oligonucleotide" does not belong to invention 1, as far as this alternative step describes the ligation of a free oligonucleotide to an immobilised nucleotide generating an immobilised nucleotide, and rather belongs to invention 4 as defined below.

invention 4

The subject-matter of claim 11 relates to a method for manufacture of a nucleic acid molecule that can be resumed in a method wherein
a) a first free ligation product (free oligonucleotide) is provided
b) a second free ligation product (free oligonucleotide) is provided
c) the first and the second oligonucleotides are cut, generating first and second free elongated ligation product
d) and e) the free elongated ligation products are ligated to further oligonucleotides, providing free ligation products
f) the ligation products are immobilised
g) and h) the immobilised ligation products are cut
l) a first released oligonucleotide (i.e. free) oligonucleotide is ligated to the second

immobilised oligonucleotide.

According to step d), the ligation of cut ligation products to the double-strand oligonucleotide is performed **in solution**. This is clearly different from the technical teaching of D1 (see for example fig.1), wherein any ligation step is actually a **solid phase** ligation step. Therefore, novelty is acknowledged (Art.33(2) PCT).
There is no technical teaching provided in the prior art and particularly not in D1 suggesting to perform the ligation step in solution rather than a solid phase ligation. Thus, inventive activity is acknowledged (Art.33(3) PCT).

invention 5

The subject-matter of claim 19 relates to a method for manufacture of a nucleic acid molecule that can be resumed in a method wherein
a) a first immobilised ligation product is provided
b) a second immobilised ligation product is provided
c) the product of a) is cut, providing a first cut immobilised ligation product
d) the product of b) is cut, providing a second immobilised ligation product (the term "first" employed is considered to have been wrongly written)
e) the product of c) is cut, providing a free double-strand DNA fragment
f) the product of e), namely a free oligonucleotide is ligated to the product of d), namely an immobilised oligonucleotide.

Thus, the subject-matter of claim 19 differs from D1 resides in the fact that the first ligation product is actually cut twice, namely in steps c) and e) thus providing for a released double-strand DNA fragment. Novelty is therefore acknowledged (Art 33(2) PCT).
Said released double-strand DNA fragment has two free ends, whereas the cut immobilised second ligation product has only one free end. This allows "flipping" the orientation of the double-stranded DNA fragment provided by the first ligation product between two consecutive assembly rounds. Insofar, the cut second ligation product can only be ligated to the cut ligation product having the proper sequence thus discriminating or excluding from ligation those ligation products of earlier assembly rounds where the preceding ligation has not been completed. As a consequence, the overall yield of the nucleic acid molecule to be synthesised is increased. Inventive

activity is therefore recognized (Art.33(3) PCT).

International patent application PCT/EP03/11551
Sloning BioTechnology GmbH
our ref: S 10010 PCT

NEW CLAIMS

1. A method for the manufacture of a nucleic acid molecule comprising the steps of

a) providing a first at least partially double-stranded oligonucleotide which has a modification allowing the oligonucleotide to be coupled to a surface, whereby the oligonucleotide comprises a recognition site for a first type IIS restriction enzyme which cuts outside its recognition site, and which oligonucleotide comprises a single-stranded overhang,

b) providing a second at least partially double-stranded oligonucleotide whereby the oligonucleotide comprises a recognition site or a part thereof or a sequence which is complementary thereto, for a second type IIS restriction enzyme which cuts outside its recognition site, and which second oligonucleotide comprises a single-stranded overhang,

c) ligating the first and the second oligonucleotide via their overhangs generating a first ligation product,

d) immobilising the first ligation product to the surface via the modification,

e) cutting the immobilised ligation product with the first type IIS restriction enzyme thus releasing an elongated oligonucleotide having an overhang,

f) combining the elongated oligonucleotide with a further at least partially double-stranded oligonucleotide which has a modification allowing the oligonucleotide to be coupled, to a surface, whereby the further oligonucleotide comprises a recognition site for a further type IIS restriction enzyme which cuts outside its recognition site and which oligonucleotide comprises a single-stranded overhang, and ligating the elongated second oligonucleotide and the further at least partially double-stranded oligonucleotide via their overhangs forming a further ligation product,

g) immobilising the further ligation product to a surface via the modification,

h) cutting the further ligation product with the further type IIS restriction enzyme releasing an elongated oligonucleotide having an overhang, and

i) optionally, repeating steps f) to h).

2. A method for the manufacture of a nucleic acid molecule comprising the steps of

a) providing a first at least partially double-stranded oligonucleotide which has a modification allowing the oligonucleotide to be coupled to a surface, whereby the oligonucleotide comprises a recognition site for a first type IIS restriction enzyme which cuts outside its recognition site, and which oligonucleotide comprises a single-stranded overhang,

b) immobilising the first oligonucleotide to the surface via the modification,

c) providing a second at least partially double-stranded oligonucleotide whereby the oligonucleotide comprises a recognition site or a part thereof for a second type IIS restriction enzyme which cuts outside its recognition site, and which second oligonucleotide comprises a single-stranded overhang,

d) ligating the first and the second oligonucleotide via their overhangs generating a first ligation product,

e) cutting the immobilised ligation product with the first type IIS restriction enzyme thus releasing an elongated oligonucleotide having an overhang,

f) providing a further at least partially double-stranded oligonucleotide which has a modification allowing the oligonucleotide to be specifically coupled to a surface, whereby the oligonucleotide contains a recognition site for a further type IIS restriction enzyme and a single-stranded overhang,

g) immobilising the further at least partially double-stranded oligonucleotide on a surface via its modification,

h) combining the elongated oligonucleotide with the immobilised further oligonucleotide, and ligating them via their overhangs forming a further ligation product,

i) cutting the resulting further ligation product with the further type IIS restriction enzyme releasing an elongated oligonucleotide having an overhang, and

j) optionally, repeating steps f) to i).

3. A method for the manufacture of a nucleic acid molecule comprising the steps of

a) providing a first at least partially double-stranded oligonucleotide which has a modification allowing the oligonucleotide to be coupled to a surface, whereby the oligonucleotide comprises a recognition site for a first type IIS restriction enzyme which cuts outside its recognition site, and which oligonucleotide comprises a single-stranded overhang,

b) providing a second at least partially double-stranded oligonucleotide whereby the oligonucleotide comprises a recognition site or a part thereof or a sequence which is complementary thereto, for a second type IIS restriction enzyme which cuts outside its recognition site, and which second oligonucleotide comprises a single-stranded overhang,

c) ligating the first and the second oligonucleotide via their overhangs generating a first ligation product,

d) cutting the ligation product with the first type IIS restriction enzyme thus generating an elongated oligonucleotide having an overhang and a shortened first oligonucleotide,

e) immobilising the shortened first oligonucleotide on a surface via the modification,

f) providing a further at least partially double-stranded oligonucleotide which has a modification allowing the further oligonucleotide to be coupled to a surface, whereby the further oligonucleotide comprises a recognition site for a further type IIS restriction enzyme which cuts outside its recognition site and which oligonucleotide comprises a single-stranded overhang.

g) combining the elongated oligonucleotide with the further oligonucleotide and ligating the elongated oligonucleotide and the further oligonucleotide via their overhangs forming a further ligation product,

h) cutting the further ligation product with the further type IIS restriction enzyme generating an elongated oligonucleotide having an overhang and a shortened further oligonucleotide, and

i) optionally, repeating steps e) to h).

4. The method according to any of claims 1 to 3, wherein the overhang is a 5′-overhang or a 3′-overhang.

5. The method according to any of claims 1 to 4, wherein the overhang is selected from the group comprising a one nucleotide overhang, a two nucleotides overhang, a three nucleotides overhang, a four nucleotides overhang, a five nucleotides overhang, a six nucleotides overhang and a seven nucleotides overhang.

6. The method according to any of claims 1 to 5, wherein the elongated oligonucleotide is transferred to a new reaction vessel where it is combined with the further oligonucleotide.

7. The method according to any of claims 1 to 6, wherein the at least partially double-stranded oligonucleotide comprises a constant region and a variable region whereby the constant region contains a recognition site for a type IIS restriction enzyme, and the variable region

contains a nucleic acid sequence which corresponds to a part of the nucleic acid sequence of the nucleic acid molecule to be manufactured.

8. A method for the synthesis of a nucleic acid molecule comprising the following steps:

a) Providing a first ligated elongated oligonucleotide by

i) providing a first elongated oligonucleotide, whereby the first elongated oligonucleotide is preferably the elongated oligonucleotide according to the method of any of claims 1 to 7;

ii) providing a second elongated oligonucleotide, whereby the second elongated oligonucleotide is preferably generated starting from the further ligation product according to the method of any of claims 1 to 7 by cutting the further ligation product by the second type IIS restriction enzyme;

iii) ligating the first and the second elongated oligonucleotide, whereby either the first and the second elongated oligonucleotides are ligated in solution and are subsequently immobilized to a surface by means of the modification, or the second elongated oligonucleotide is immobilized to a surface by means of the modification and subsequently the first elongated oligonucleotide is ligated thereto generating in both cases a first ligated elongated oligonucleotide,

b) providing a second ligated elongated oligonucleotide by

i) providing a third elongated oligonucleotide, whereby the third elongated oligonucleotide is the elongated oligonucleotide according to the method of any of claims 1 to 7;

iv) providing a fourth elongated oligonucleotide, whereby the fourth elongated oligonucleotide is generated starting from the further ligation product according to the method of any of claims 1 to 7 by cutting the further ligation product by the second type IIS restriction enzyme:

v) ligating the third and the fourth elongated oligonucleotide, whereby either the third and the fourth elongated oligonucleotides are ligated in solution and subsequently immobilized to a surface by means of the modification, or the fourth elongated oligonucleotide is immobilized to a surface by means of the modification and subsequently the third

elongated oligonucleotide is ligated thereto generating in both cases a second ligated elongated oligonucleotide,

c) cutting the first ligated elongated oligonucleotide by a type IIS restriction enzyme, whereby the restriction enzyme is the first type IIS restriction enzyme, generating a first cut ligated elongated oligonucleotide;

d) cutting the second ligated elongated oligonucleotide by a type IIS restriction enzyme, whereby the restriction enzyme is the second type IIS restriction enzyme, generating a second cut ligated elongated oligonucleotide;

e) combining and ligating the first cut ligated elongated oligonucleotide and the second cut ligated elongated oligonucleotide;

f) optionally repeating steps a) to e), whereby the ligation product of step e) is used as a first ligated elongated oligonucleotide and/or as a second ligated elongated oligonucleotide .

9. A method for the manufacture of a nucleic acid molecule comprising the steps of

a) providing a first at least partially double-strandedoligonucleotide which has a modification allowing the oligonucleotide to be coupled to a surface, whereby the oligonucleotide comprises a recognition site for a first type IIS restriction enzyme which cuts outside its recognition site, and which oligonucleotide comprises a single-stranded overhang, and whereby the oligonucleotide comprises a part of the nucleic acid molecule to be manufactured,

b) immobilizing the first oligonucleotide on a surface

c) cutting the first oligonucleotide with the first type IIS restriction enzyme releasing a double stranded oligonucleotide having a single stranded overhang at each end and being a part of the nucleic acid molecule to be manufactured,

d) combining the double stranded oligonucleotide of step c) with a second at least partially double-stranded oligonucleotide which has a modification allowing the oligonucleotide to be coupled to a surface, whereby the oligonucleotide contains a recognition site for a second type IIS restriction enzyme which cuts outside its recognition site, and which oligonucleotide further comprises a single-stranded overhang and a part of the nucleic acid molecule to be manufactured, and ligating the double-stranded oligonucleotide of step c) with the second oligonucleotide;

whereby the overhang of the second oligonucleotide is essentially complementary to the overhang of the double stranded oligonucleotide of step c).

10. The method according to claim 9, wherein the overhang generated upon cutting the first oligonucleotide with the first type IIS restriction enzyme is essentially complementary to the overhang of the second at least partially double stranded oligonucleotide.

11. A method for the manufacture of a nucleic acid molecule comprising the following steps:

a) providing a first ligation product, whereby the first ligation product consists of a first oligonucleotide moiety comprising a recognition site for a first type IIS restriction enzyme, a second oligonucleotide moiety comprising a recognition site for a second type IIS restriction enzyme and a third oligonucleotide moiety, whereby the third oligonucleotid moiety is a part of the nucleic acid molecule to be manufactured, and whereby the first and the second type IIS restriction enzymes each generate an overhang, whereby the overhang generated by the first type IIS restriction enzyme has a length which is different from the length of the overhang generated by the second type IIS restriction enzyme;

b) providing a second ligation product, whereby the second ligation product consists of a first oligonucleotide moiety comprising a recognition site for a third type IIS restriction enzyme, a second oligonucleotide moiety comprising a recognition site for a fourth type IIS restriction enzyme and a third oligonucleotide moiety, whereby the third oligonucleotide moiety is a part of the nucleic acid molecule to be manufactured, and whereby the third and the fourth type IIS restriction enzyme each generate an overhang, whereby the overhang generated by the third type IIS restriction enzyme has a length which is different from the length of the overhang generated by the fourth type IIS restriction enzyme;

c) cutting the first ligation product with the second restriction enzyme generating a first cut ligation product and cutting the second ligation product with the fourth restriction enzyme generating a second cut ligation product;

d) providing a third at least partially double-stranded oligonucleotide and ligating the third oligonucleotide with the first cut ligation product, whereby the third oligonucleotide comprises an overhang which is complementary to the overhang of the first cut ligation product generated in step c) and whereby the third oligonucleotide comprises a recognition site for a fifth IIS restriction enzyme;

e) providing a fourth at least partially double-stranded oligonucleotide and ligating the fourth oligonucleotide to the second cut ligation product, whereby the fourth oligonucleotide comprises an overhang which is complementary to the overhang of the second ligation product generated in step c) and whereby the fourth oligonucleotide comprises a recognition site for a sixth type IIS restriction enzyme;

f) immobilising the ligation product of step d) and step e) on a surface by means of a modification of the third oligonucleotide and the fourth oligonucleotide;

g) cutting the immobilised ligation product of step d) with the fifth type IIS restriction enzyme releasing an oligonucleotide;

h) cutting the immobilised ligation product of step e) with the third type IIS restriction enzyme; and

i) combining and ligating the oligonucleotide released according to step g) with the immobilised reaction product of step h),

whereby the overhang generated by the first and the third restriction enzyme is complementary to the overhang generated by the fifth and sixth restriction enzyme.

12. The method according to claim 11, wherein the first and the third restriction enzyme are identical and/or the second and the fourth restriction enzyme are identical and/or the fifth and the sixth restriction enzyme are identical.

13. The method according to claim 11 or 12, wherein the first and the third restriction enzyme and the fifth and the sixth restriction enzyme are each a restriction enzyme generating a four nucleotide overhang, preferably at the 5' end.

14. The method according to any of claims 11 to 13, wherein the second and the third restriction enzyme is a restriction enzyme creating an overhang having a length which is selected from the group comprising 1, 2, 3, 4, 5 and 6 nucleotides.

15. The method according to any of claims 11 to 14, wherein the first and the second restriction enzyme is Esp3I or Eco31I and the fifth and the sixth restriction enzyme is Ecp31I or Esp3I.

16. The method according to any of claims 11 to 15, wherein the ligation product of step i) is used as a first ligation product and/or a second ligation product and steps a) to i) are repeated one or several times.

17. The method according to any of claims 11 to 16, wherein the third moiety is arranged between the moieties of the oligonucleotides containing the restriction site for the type IIS restriction enzymes.

18. The method according to any of claims 11 to 17, wherein the first and the second ligation products are provided in separate reaction vessels.

19. A method for the manufacture of a nucleic acid molecule comprising the following steps:

a) Providing a first ligation product, whereby the first ligation product consists of a first oligonucleotide moiety comprising a recognition site for a first type IIS restriction enzyme, a second oligonucleotide moiety comprising a recognition site for a second type IIS restriction enzyme and a third oligonucleotide moiety, and immobilising the first ligation product via a modification to a surface, whereby the modification is incorporated by the second moiety,

b) providing a second ligation product, whereby the second ligation product consists of a first oligonucleotide moiety comprising a recognition site for a first type IIS

restriction enzyme, a second oligonucleotide moiety comprising a recognition site for a second type IIS restriction enzyme and a third oligonucleotide moiety, and immobilising the second ligation product via a modification to a surface, whereby the modification is incorporated by the second moiety,

c) cutting the first ligation product with the restriction enzyme the recognition site of which is contained in the first moiety providing a cut immobilised first ligation product,

d) cutting the second ligation product with the restriction enzyme the recognition site of which is contained in the first moiety providing a cut immobilised second ligation product,

e) cutting the cut immobilised first ligation product with the restriction enzyme the recognition site of which is contained in the second oligonucleotide moiety releasing a double-stranded DNA fragment,

f) combining and ligating the double-stranded DNA fragment with the cut immobilised second ligation product.

20. The method according to claim 19, wherein the ligation product of step f) is combined and ligated with an elongated oligonucleotide according to any of the preceding claims, whereby this ligation product is used as a first or a second ligation product in step a) or step b) in the method of claim 19.

21. The method according to claim 19 or 20, wherein the DNA fragment is the nucleic acid molecule or part thereof which is to be manufactured.

22. The method according to any of claims 19 to 21, wherein the third moiety is arranged between the moieties of the oligonucleotides containing the restriction site for the type IIS restriction enzymes.